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Qualitative and quantitative assessment of bioactive compounds present in sponge gourd (*Luffa aegyptica*) fruit pulp

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Abstract
This research work examined the qualitative and qualitative phytochemical composition of sponge gourd (*Luffa aegyptica*) fruit pulp, to ascertain the bioactive compounds present in the fruit. Ethanolic extract of the fruit was subjected to various tests to identify the presence of alkaloids, tannins, saponins, glycosides, polyphenols, steroids, phlobatanins and anthraquinones. Results revealed the presence of all bioactive compounds investigated except phlobatanins and reducing compound which were not detected. The active compound with the highest amount was Alkaloid (24.3%), followed by glycosides (12.6%), flavonoid (11.2%), saponins (10.3%), Tannins (6.4%) and phenol (3.5%). This implies that the consumption of the fruit can help in treatment of inflammation. It is a good medicinal plant that should be encourage in production of pharmaceutical products.

Keywords: Sponge gourd, *Luffa aegyptica*, phytochemicals

Introduction

Medicinal plants have been known for decades and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of disease and aliments (Joshua and Asim, 2017) [4]. Medicinal plants have been used from ancient time for the medical values. Nowadays, the crude extracts samples from medicinal plants have been shown interest for the development and preparation of alternatives traditional medicine (Katan *et al.*, 2003) [6]. Plants are the best source for chemical ingredients or phytochemical agents for cure of different diseases.

Fig 1: Sponge gourd (L*uffa aegyptica*)

Lufffa aegyptiaca is an important plant with excellent medicinal properties. *L. aegyptiaca* is commonly called "sponge gourd" loofa, vegetable sponge, bath sponge or dish cloth guard, is a member of cucurbitaceous family (Wentzel and Hazel, 2021) [10]. The fruits of *luffa aegyptical* are smooth and Cylindrical shaped. One fruit of luffa sponge produces at least 300 seeds (Sangh and Sari, 2019) [11]. *L. aegyptiaca* has alternative and palmate leaves comprising petiole. The leaf is 13cm and 30cm in length and width respectively and has the acute end lobe. It is hairless and has serrated edges.

It has been reported that phytochemicals which are considered as secondary metabolites components are directly responsible for activity such as antimicrobial, antifungal, anticancer, anti-inflammatory among others (Vaghasiya *et al.*, 2011) [13]. Therefore, screening of chemical constituent in medicinal plant in order to assess for their availability may provide new useful information to the scientific community and in claiming for their therapeutic efficacies. There are a lot of problem of diseases that, are rising from the use of chemicals (synthesized drugs) and synthesized drugs are offering very little help in treatment of some diseases. This study therefore aimed at screening chemical constituent of *Luffa aegyptiaca* leaf sample in Calabar South Local Government Area of Cross River State in order to provide vital information on their availability.

Methodology

1. Sample collection and treatment

Sponge gourd (*luffa aegyptica*) was collected in Calabar South, Cross River State. The laboratory table washed with running tap water, followed by hydrogen hypochorite (bleach) solution to further clean and disinfect the work area. The sponge gourd fruits were washed in tap water, rinsed in distilled water and lastly in deionized water. The sample was allowed to be air dried for 30 minutes, grinded and placed in the oven to dry. The dried pulp was then weighed and stored for extraction.

2. Extraction procedure

Soxhlet extraction was carried out by infusing the powdered sample in ethanol in a stopped container for 24 hours with intermittent agitation until soluble matter was dissolved.

3. Qualitative screening method

Test for alkaloids

One gram of crude extract was prepared by dissolving 1g of the extract in 10 ml of 10% (v/v) HCl. Heated and filtered into a test tube, followed by the addition of 1.0 ml of Mayer's reagent. Formation of brown/ thick brown precipitates indicated the presence of alkaloids.

Test for saponins (frothing test)

One gram of extracts (1 g) was suspended in water in a test tube, shaken vigorously and checked for froth. It was warmed gently at 50°C for 10 minutes in water bath apparatus and shaken vigorously again. Frothing which persisted on warming was preliminary evidence of the presence of saponins.

Test for flavonoids

The extract (1 g) was treated with few drops of concentrated HCl. The formation of orange colour precipitate indicated the presence of flavonoids.

Test for steroids (Salkowaski reaction)

One milligram of the crude plant extracts was dissolved in concentrated sulphuric acid and acetic acid. A brown reddish colouration indicated the presence of steroids.

Test for glycosides (modified Bornkragers test)

One gram of the extracts was hydrolyzed with dilute concentrated sulphuric acid for glycoside, a reddish brown colourisation at the interface indicate in the presence of glycosides.

Test for tannins (ferric chloride reagent test)

The sponge gourd extract was dissolved in 20 ml of deionize water and filtered. To the test tube, 10 ml of the filtrate was added, followed by a few drops (2 - 3) of 0.1% Ferric chloride ($FeCl_3$) and glacial acetic acid solution. The mixture was examined for the formation of blue, brownish green or blue- precipitate.

Test for Anthraquiones

One milliliter (1 ml) of the sponge gourd filtrate was shaken with 10 ml of benzene. The mixture was filtered and 5ml of 10% (v/v) ammonia was added, then shaken and observed. There was no significant change, therefore, it was not detected.

Test for plobatannins

About 0.5grams of the plant extract was dissolved in distilled after and filtered. the filtrate was boiled in 2% HCl, a red precipitate showed the presence of phlobatannins,

Test for polyphenols (Potassium ferrocyandie test)

A quantity (2ml) of the ethanolic extract was added to 5 ml of distilled water and heated for 30 minutes in a water bath. It is was followed by addition of 1 ml of 1% $FeCl_3$ followed by 1 ml of 1% potassium ferrocyanide solution. The formation of green colouration indicated the presence of polyphenol.

Reducing compound

Ferric solution was added to 1 ml of the extract. The test showed no significant change. Therefore, the presence of reducing compound was not detected.

4. Quantitative Phytochemical Screening

Determination of total alkaloids

One gram of the sample was weighed into a 250 ml beaker, 20 ml of 10% acetic acid in ethanol was added, filtered and allowed to stand for 4 hours. To one-quarter of the original volume, concentrated ammonium hydroxide was added slowly to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue was alkaloid, which was dried and weighed.

Determination of total Tannis

Five hundred milligram of the sample was weighed into a 500 g plastic sieve. 50 ml of distilled water was added and shaken in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark with distilled water. Then 10 ml of the filtrate was pipetted into a test tube mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1M HCL and 0.008 M potassium ferrcynanide. Absorbance was measured at 410 within 10 minutes.

Determination of total Saponins

Twenty grams of the grinded sample was placed in a conical flask and 100 cm^3 of 20% aqueous ethanol was added. Heat was applied to a hot water bath for 4 hours with continuous stirring at 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml 20% ethanol. The extracts were combined and reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethylether was added and the resulting mixture was shaken vigorously. The aqueous layer was collected while the either layer was discarded. Purification process was repeated by adding 60 ml of n-butanol, washing off the combined n-butanol extracted juice with 10ml of 5% aqueous sodium chloride. Heating of the remaining solution was carried was oven dried and constant weight was taken to determine the amount of saponins.

Determination of total Flavonoids

The sample was repeatedly extracted with 100 ml of aqueous methanol at room temperature. The extract was filtered with a filter paper and the filtrate was later transferred into a water bath and solution was heated to dryness. The resulting mixture was then weighed.

Determination of total Phenolic compounds

The total quantity of phenol was determined using the spectrophotometer method. The plant sample was boiled 150 ml of ethanol for 15 minutes. To 5 ml of the boiled sample in a 5 ml flask, 10 ml of distilled water was added followed by 2ml of NH_4 solution and 5ml of concentrated ethyl alcohol. The mixture was left to react for 30 minutes for colour development and measured at 505nm wavelength using a spectrophotometer.

3. Results and discussion

The results of the qualitative phytochemical analysis of *luffa aegyptica* are presented in Table 1. The bioactive compounds reported in the sample are known to exhibit medicinal qualities, while he results of the quantitative analysis of the secondary metabolic in sponge gourd sample are shown in table 2. Results revealed that alkaloids had the highest percentage composition of 24.3% while phenols had

the lowest percentage of 3.5%, others include glycosides (12.6%), flavonoids (11.2%), tannins (6.4%) and saponins (10.3).

Table 1: Qualitative Phytochemical screening of sponge gourd (*luffa aegyptica*) fruit pulp

Component	Presence
Alkanoids	+++
Saponins	++
Flavonoids	++
Glycosides	++
Polyphenols	+++
Anthraquinones	+++
Phlobatannis	ND
Stenoids	+
Tannis	+++
Reducing compound	ND

+ = Slightly present
++ = Moderably present
+++ = Heavily present
ND = Not detected

Table 2: Quantitative phytochemical screening of (*luffa aegyptica)* fruit pulp

Parameter (%)	% Proportion
Alkaloids	24.3
Saponins	10.3
Tannins	6.4
Flavonoids	11.2
Phenols	3.5
Glycoside	12.6

The findings obtained in this study agrees with the results presented by Omoboyowa *et al.,* (2015) [9] who reported alkaloid (24.0) phenol (3.5/100g), flavonoids (11.0/100g) tannins (6.2/ 100g) and saponins (10.6/100g)m for methanol extract of *luffa aegyptica*. Gwana *et al.,* (2014) [5] also reported phytochemical percentage composition of sponge gourd to contain alkaloid (23.4%), phenol (3.6)%, flavonoids (11.4%), tannin (6.2%), and saponins (10.4%).

The major activity of medicinal plants against ailments is a function of the amount of secondary metabolite. However, the scope of this research work did not cover the investigations of the specific roles of the extracted phytochemicals from sponge gourd. It has however, been reported from studies that the secondary metabolism is responsible for many pharmacological actions of plants and vegetables.

There are several physiological properties of alkaloids such as anti-inflammatory, anti-bacterial and analgesic, etc. The presence of alkaloid showed that sponge gourd can be used as basic medicinal agents for analgesic and bactericidal effects (Okwu *et al.,* 2004) [8]. Studies have shown that alkaloid are known to have an effect on the central nervous system and acts as a pain killer, such as morphine (Eleazu *et al.,* 2010).

The presence of saponins is an indication that the plants possess the property of triggering and clotting red blood cells. Some of the characteristics include formation of foams in aqueous solutions, hemolytic, activity cholesterol binding properties and bitterness (Omoboyowo *et al.,* 2015). Phytosterols are one of the many nutrients that are be heart healthy, 2-3 grams of phytosterols per day, for 3-4 weeks, can reduce low density lipoprotein (LDL) cholesterol by around 10% (Katan *et al.,* 2013) [6].

Phenol compounds have been reported to service antioxidants and exhibit a wide range spectrum of medicinal properties such as anticancer, anti-inflammatory, diabetes, etc. The phytochemicals also act as antioxidants which inhibits free radicals and thereby weaken inflammatory process (Abdelwahas *et al.,* 2011) [1].

Dutta *et al.,* (2003) [2] reported that, tannins are effective in the treatment of skin diseases. Tannis astringent properties help in the treatment of intestinal disorder. Tannis are dietary anti-nutrient that are responsible for the food processing industries such as manufacturing of wine. The treatment of score throat and wound healing has also been linked to tannins, but if ingested in excessive quantities, the excessive absorption of mineral such as iron and calcium which may lead to anemia if prolonged (Tiwari *et al.,* 2011).

Various bioactive properties such as anticancer, anti-inflammatory and anti-allergic abilities by flavonoids have been reported (Okwu *et al.,* 2014) [8], flavonoids are good antioxidants and free radical scavengers which help in inhibiting cancer cell activity. They can lower the risk of arthritis, allergies and viral diseases caused by herpes sample virus, parainflenza virus and adenovirus (Okwu *et al.,* 2014) [8].

Plants terpenoids are used extensive for their aromatic qualities and play a role in traditional herbal remedies as they are used as flavour enhancer. Terpenoids exhibit various important pharmacological activities i.e anti-inflammatory, anticancer, anti-malaria, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities.

Conclusion

This study has shown that sponge gourd which are often considered waste portion of the fruit are good source of phytochemicals that are biologically important. Hence, their consumption and usage in medicine in further encourage. Sponge gourd (*Luffa agypetica*) is a good medicinal plant, it should be encouraged and used for pharmaceutical production, hence the consumption and usage in herbal medicine is further encouraged.

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